IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re: Appeal to the Board of Patent Appeals and Interferences

PATENT APPLICATION

In re PATENT APPLICATION of
Inventor(s): Masahiro IWAMOTO *et al.*
Appln. No.: 09 (Series Code ↑) 902,772 (Serial No. ↑)
Filed: July 12, 2001
Title: CELL CALCIFICATION SUPPRESSING PROTEINS AND GENES OF THE PROTEINS

Group Art Unit: 1653
Examiner.: Schnizer, Holly G.
Atty. Dkt. 046124 | 5001-US01

Date: March 23, 2004

Commissioner for Patents
Arlington, VA 22202

Sir:

1. ☒ **NOTICE OF APPEAL**: Applicant hereby appeals to the Board of Patent Appeals and Interferences from the decision (not Advisory Action) dated September 23, 2003 of the Examiner twice/finally rejecting claim(s) in this application or in this application and its parent application.
2. ☐ **BRIEF** on appeal in this application attached in triplicate (extendable up to 5 months).
3. ☐ An **ORAL HEARING** is respectfully requested under Rule 194 (due two months after Examiner's Answer- unextendable)
4. ☐ Reply Brief is attached in triplicate (due two months after Examiner's Answer – unextendable).
5. ☐ "Small entity" statement filed: ☐ herewith. ☐ previously.

6. ☐ Fee NOT required if/since paid in prior appeal in which the Board of Patent Appeals and Interferences did not render a decision on the merits (35 USC 134).

7. **FEE CALCULATION**

			Large/Small Entity		Fee Code
If box 1 above is X'd, ... enter			$330/165	$330	119/219
If box 2 above is X'd, ... enter			$330/165	$0	120/220
If box 3 above is X'd, ... enter			$290/145	$0	121/221
If box 4 above is X'd, ... enter nothing			- 0 - (no fee)		
8. Original due date: November 25, 2003					
9. Petition is hereby made to extend the original due date	(1 mo)	$110/$55	$		115/215
to cover the date this response is filed for which the	(2 mos)	$420/$210			116/216
requisite fee is attached.	(3 mos)	$950/$475	$950		117/217
	(4 mos)	$1,480/$740			118/218
(Usable only if box 2 is X'd--- 5 mos)		$2,010/$1,005	+$0		128/228
10. Enter any previous extension fee paid ☐ previously since above Original due date (item 8); ☒ with concurrently filed amendment.....			$950		
11. **Subtract line 9 from line 8 and enter: Total Extension Fee**				+$	
12. **TOTAL FEE ATTACHED =**				$330	

(Our Deposit Account No. **50-0310**)
(Our Order No. 046124 (C#) | 5001-US01 (M#))

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 (missing or insufficiencies only) now or hereafter relative to this application and the resulting Official Document under Rule 20, or credit any overpayment, to our Accounting/Order Nos. shown above, for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal sheet is filed.

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NOTE: File this cover sheet in duplicate with PTO receipt (PAT-103A) and attachments

03/24/2004 YPOLITE1 00000104 500310 09902772

01 FC:1401 330.00 DA

Invitrogen
life technologies

Lipofectamine™ Reagent

Cat. No.: 18324-012
Conc.: 2 mg/ml

Size: 1 ml
Store at 4°C.
***DO NOT FREEZE.* MIX GENTLY BEFORE USE.**

Description:
Lipofectamine™ Reagent is a 3:1 (w/w) liposome formulation of the polycationic lipid 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) (Chemical Abstracts Registry name: N-[2-({2,5-bis[(3-aminopropyl)amino]-1-oxypentyl}amino) ethyl]-N,N-dimethyl-2,3-bis(9-octadecenyloxy)-1-propanaminium trifluoroacetate), and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water. It is suitable for the transfection of DNA into cultured eukaryotic cells (1). One milliliter is sufficient for 50 to 200 transfections on 35-mm tissue culture dishes or 15 to 70 transfections on 60-mm dishes. The transfection activity of Lipofectamine™ Reagent can be further enhanced by the use of Plus™ Reagent (3) to pre-complex DNA prior to the preparation of the transfection complexes (See the protocol below).

Quality Control:
1. Lipofectamine™ Reagent is tested functionally by transfecting subconfluent BHK-21 cells with pGL3 DNA. The cells are assayed for luciferase activity using a modification of the procedure of Wood (2).
2. Lipofectamine™ Reagent is tested for the absence of bacterial and fungal contamination using blood agar plates and fluid thioglycolate medium.

Protocols:
Transient or Stable Transfection of Adherent Cells: [A diagram of this procedure appears in Figure 1].
The concentrations of DNA and Lipofectamine™ Reagent, cell density and transfection times in these transfection protocols were determined with the plasmids pSV2-CAT and pCMVCAT in BHK-21, HeLa, COS-7, CHO-K1, NIH3T3, and PC12 cell lines as well as passaged primary human fibroblasts (1). Stable transformations were performed with pRSV*neo* plasmid DNA in NIH3T3 cells (1). These conditions are recommended as guidelines only. For larger tissue culture dishes, Table I shows the suggested amounts of reagents. Adjust all amounts in proportion to the change in culture dish surface area.
1. The day before transfection, seed ~2-6 × 10^4 cells per well of a 24-well plate in 0.5 ml of the appropriate complete growth medium (with serum if cells are normally cultured in the presence of serum).
2. Incubate the cells at 37°C in a CO_2 incubator until the cells are 50-80% confluent. This will usually take 18-24 hours, but the time will vary among cell types. (Optimal cell density may vary with cell type or application. Since transfection efficiency is sensitive to culture confluence, it is important to maintain a standard seeding protocol from experiment to experiment.)
3. For each well in a transfection, dilute 0.2-0.4 µg of DNA into 25 µl medium without serum. Mix gently. Opti-MEM® I Reduced Serum Medium (Cat. No. 31985) gives optimal results.
4. For each well in a transfection, dilute 0.5-5 µl of Lipofectamine™ Reagent into 25 µl medium without serum and mix.
5. Combine diluted DNA (from step 3) and diluted Lipofectamine™ Reagent (from step 4), mix gently, and incubate at room temperature for 15-45 min. to allow DNA-liposome complexes to form. The solution may appear cloudy, but this will not impede the transfection. While complexes are forming, replace the medium on the cells with 0.2 ml of transfection medium without serum. (This is usually the cell growth medium without serum). **Note:** Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test media for compatibility with transfection reagent before use.
6. For each transfection, add 0.15 ml of medium without serum to the tube containing the complexes. Mix gently and overlay the diluted complex solution onto the rinsed cells. Medium containing serum may be added to the complexes at this step (see Note 3). Do not add antibacterial agents to media during transfection.
7. Incubate the cells with the complexes for 2-24 hours at 37°C in a CO_2 incubator. Invitrogen recommends starting with 5 hours.
8. Following incubation, add 0.4 ml of growth medium containing twice the normal concentration of serum without removing the transfection mixture. (If serum was included in step 6, add 0.4 ml of complete growth medium at this time.) If toxicity is a problem, remove the transfection mixture and replace it with complete growth medium.
9. Replace the medium with fresh, complete medium at 18-24 hours following the start of transfection if continued cell growth is required.
10. Assay cell extracts for transient gene expression 24-72 hours after the start of transfection, depending on cell type and promoter activity.
11. A similar procedure can be used to transfect DNA for stable expression. At 72 hours after transfection, passage the cells 1:10 into the selective medium for the reporter gene transfected. For instance, for pRSV*neo* transfections, medium should contain Geneticin® Selective Antibiotic (Cat. No. 11811).

Enhancement of Transfection with Plus™ Reagent:
Perform steps 1 and 2 as in the protocol for Transient or Stable Transfection of Adherent Cells.
3. Pre-complex the DNA with Plus™ Reagent (Cat. No. 11514-015): Dilute 0.4 µg DNA into 25 µl dilution medium without serum (Dulbecco's Modified Eagle Medium (D-MEM) works best for Plus™ Reagent). Mix Plus™ Reagent before use. Add 4 µl Plus™ Reagent to diluted DNA, mix again, and incubate at room temperature for 15 min.
4. Dilute 1 µl Lipofectamine™ Reagent into 25 µl dilution medium without serum in a second tube; mix.
5. Combine pre-complexed DNA (from step 3) and diluted Lipofectamine™ Reagent (from step 4); mix and incubate for 15 min at room temperature.
6. While complexes are forming, replace the medium on the cells with 0.2 ml of transfection medium without serum (cell growth medium without serum). **Note:** It is possible to include serum in the transfection medium at this step. **Note:** Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test media for compatibility with transfection reagent before use.
7. Add the DNA- Plus™-Lipofectamine™ Reagent complexes (from step 5) to each well of cells containing fresh medium. Mix complexes into the medium gently; incubate at 37°C at 5% CO_2 for 3 hours.
8. After 3 h incubation, increase volume of medium to normal volume; add serum to bring the final concentration to that of normal growth medium. If necessary to maximize cell growth, replace the medium containing the complexes with fresh, complete medium after 3 h incubation or the day after transfection.

Continue protocol by following steps 9 to 11 above.

Figure 1. Diagram of Lipofectamine transfection procedure for 35-mm wells.

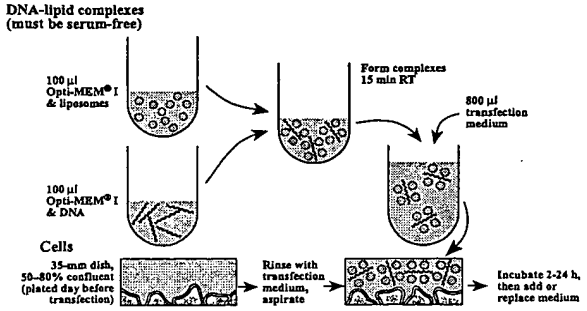

Table I. Reagent Quantities for different sized culture vessels

Culture plate diameter (mm)	Lipid and DNA dilution volume (µl)	Lipofectamine™ volume range (µl)	DNA amount (µg)	Transfection medium volume (ml)
35	100	2-25	1-2	0.8
60	300	6-75	3-6	2.4
100	800	16-200	8-16	6.4

***Transfection Optimization*:**

Careful optimization of transfection conditions is essential for the highest efficiency transfections and lower toxicity. The conditions that should be optimized include lipid and DNA concentrations, cell number, and time of exposure of cells to DNA-liposome complexes. To optimize the amount of Lipofectamine™ Reagent start with 80% confluent cells and 0.2-0.5 µg DNA for 24-well tissue culture plates and a 5-hour exposure time. With cell number, DNA concentration, and exposure time held constant, vary the amount of Lipofectamine™ Reagent to determine the optimal concentration (usually 0.5-5 µl). The cell number, amount of DNA, and time of exposure of cells to the complexes (2-24 hours) can also be optimized. Examples of the effects of varying cell number, lipid, and DNA concentrations on transfection of pCMVCAT DNA into BHK-21 cells are shown in Figures 2 and 3 below. Transfections were for 5-6 hours in 35-mm wells of cells plated the day before transfection in both cases. The upper sections of the graphs show the percent of total extractable protein recovered in the transfected samples as compared to a control sample. This protein profile correlates directly with the percent control cells present at the time of harvest and is shown as an indication of cell yield. Lower cell yield results from a decrease in the rate of cell division or toxicity and is observed associated with effective transfection by most transfection methods. The amount of CAT activity detected in a standard volume of extract is shown in the lower sections of the graphs. It is possible to increase cell yield by increasing the number of cells plated per well or by decreasing either Lipofectamine™ Reagent or DNA concentrations. With careful optimization, this can be achieved with little impact on the level of transgene expression.

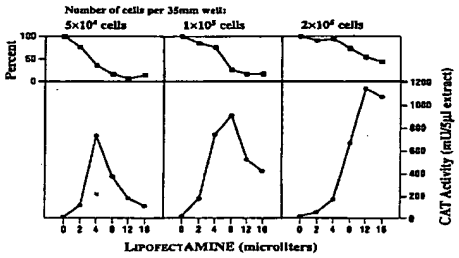

Figure 2. Effect of cell number and lipid concentration on transfection activity and protein yield. Cells were transfected with 1 µg pCMVCAT DNA (1).

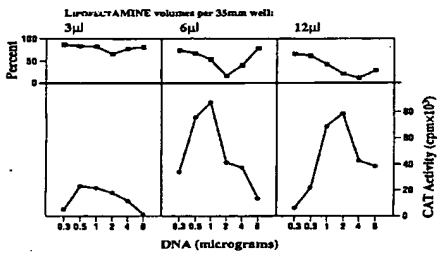

Figure 3. Effect of DNA and lipid concentrations on transfection activity and protein yield. 1×10^5 cells were transfected with pCMVCAT DNA (1).

Notes:

1. Lower cell yield or toxicity is often associated with transfection activity regardless of transfection method. To increase cell yield, transfect cultures at a higher confluence, use less Lipofectamine™ Reagent or DNA in the transfection, or include serum during exposure of cells to DNA-liposome complexes (see Note 3).
2. Do not add antibacterial agents to media during transfection.
3. If the cells will not tolerate the absence of serum for 2-24 hours, it is possible to transfect them in the presence of serum. This is done by preparing DNA-liposome complexes for 45 min in serum-free medium, followed by diluting the complexes with serum-containing medium before adding to the cells. It is extremely important that the amount of lipid be re-optimized as the optimal amount of lipid under these circumstances may be different from that observed for serum-free transfection.
4. Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test any new serum-free formulation for compatibility with the transfection reagent prior to use. Media formulations that have been found to inhibit transfections are: CD 293 Medium, 293 SFM II, and VP-SFM.

References:

1. Hawley-Nelson, P., Ciccarone, V., Gebeyehu, G., Jessee, J., and Felgner, P.L., (1993) *Focus® 15*, 73.
2. Wood, K. V., (1991) in *Bioluminescence and Chemiluminescence: Current Status*, ed. P. Stanley and L. Kricka, John Wiley and Sons, Chinchester, p. 543.
3. Shih, P., Evans, K., Schifferli, K., Ciccarone, V., Lichaa, F., Masoud, M., Lan, J., and Hawley-Nelson, P. (1997) *Focus® 19*, 52.

For references and protocols pertaining to transfection of your cell type, please see our web site at http://www.invitrogen.com/transfection/celltypes/

Paper No. 29

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte STEVEN C. CLARK, GORDON G. WONG,
PAUL SCHENDEL, AND JOHN MC COY

Appeal No. 2001-2308
Application No. 07/704,578

ON BRIEF

Before WILLIAM F. SMITH, MILLS, and GRIMES, Administrative Patent Judges.

WILLIAM F. SMITH, Administrative Patent Judge.

DECISION ON APPEAL[1]

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 28 and 29. Claims 28 and 29 read as follows:

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[1] The Examiner's Answer was mailed on May 4, 1994. For reasons not clear from the record, the file was not forwarded to the Board until July 2001. In view of the delay in the case being forwarded to the Board, we have taken the case up for decision out of turn.

28. A pharmaceutical composition which comprises an effective amount of a polypeptide having the sequence:

Ala Pro Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn MET Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys MET Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln MET Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp MET Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln MET

in combination with a pharmaceutically acceptable vehicle, wherein said polypeptide is non-glycosylated.

29. A bacterially produced non-glycosylated protein substantially free of other protein and characterized by the amino acid sequence:

Ala Pro Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn MET Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys MET Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln MET Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp MET Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln MET

The examiner relies on the following references:

Hirano et al (Hirano) "Purification to homogeneity and Characterization of Human B-Cell Differentiation Factor (BCDF or BSFp-2)," Proc. Natl Acad. Sci Vol. 82. pp. 5490-5494 (1985)

Weissenbach et al (Weissenbach), "Two Interferon mRNAs in Human Fibroblasts: In Vitro Translation and Escherichia Coli Cloning Studies," Proc. Natl. Acad. Sci Vol. 77, pp. 7152-7156 (1980)

Gilbert et al. (Gilbert),		4,338,397	Jul. 6 1982
Kishimoto (Japanense Patent)	JP	61-115025	Jun 02, 1986

Zilberstein et al (Zilberstein), "Human Interferon-β_2: Is It An Interferon-Inducer?," The Interferon System Serono Symposia Vol. 24. pp. 73-83 (1985)

The examiner also relies upon three additional documents without providing bibliographic information; based on the file history, we deduce these to be:

Revel et al (Revel) (Great Britian)	2,063,882	Nov. 19, 1990
Clark et al (Clark)	4,675,285	Jun 23, 1987
Ingolia	4,559,302	Dec. 17, 1985

GROUNDS OF REJECTION[2]

1. Claims 28 and 29 stand rejected alternatively under 35 U.S.C. 102 or 35 U.S.C. 103 as anticipated or obvious. As evidence of anticipation or obviousness, the examiner cites Hirano, Weissenbach, or Zilberstein.

2. Claims 28 and 29 stand rejected under 35 U.S.C. 103. As evidence of obviousness, the examiner cites Hirano, Weissenbach, Zilberstein, Revel, or JP 115025A in view of Gilbert, Ingolia, or Clark.

We reverse.

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[2] A final rejection of claim 28 under 35 U.S.C. §112, first paragraph (enablement) and 35 U.S.C. §101 (utility) was withdrawn in the examiner's answer.

Background

Interleukin-6 (IL-6) is a natural substance having many biological activities. Many different names have been given to the natural substance by researchers obtaining the protein from a variety of different sources: 26 KD protein, IFN-beta-2, BCDF, and BSF-2, for example (specification, page 2). A cDNA sequence encoding IL-6 was isolated from an HTLV-transformed T cell line (specification, page 12). The cDNA is 1.1 kb long, and contains an open reading frame of 636 nucleotides encoding a protein of 212 amino acids including a leader secretory sequence (specification, page 8). The invention at bar involves the protein sequence from amino acids 28 through 212 (specification, page 7, and Figure 1). The disclosure includes production of glycosylated IL-6 in mammalian cell lines. The protein produced in a mammalian cell line has an apparent molecular weight range of approximately 20 to 35 KD, indicative of glycosylation (e.g. specification, page 21). Importantly, the claims before us are limited to the nonglycosylated protein. Production of nonglycosylated IL-6 in bacterial cells is disclosed, for example, at specification pages 6, 7, 18-20.

Rather than recapitulating the arguments of appellant and examiner, we refer to pages 11-33 of the Brief for the appellant's position, and pages 4-15 of the Examiner's Answer for the examiner's position.

Rejection 1

Claims 28 and 29 stand rejected alternatively under 35 U.S.C. §102 or 35 U.S.C. §103 as anticipated by or obvious over Hirano, Weissenbach, or Zilberstein.

Under certain circumstances, the USPTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. For example, where the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the USPTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product[3]. However, the examiner bears the initial burden of providing facts and reasons to believe that the prior art products are identical or substantially identical to the claimed product.

Claim 28 requires a nonglycosylated protein in combination with a pharmaceutically acceptable vehicle. In reviewing the Examiner's Answer, nowhere do we find that the examiner has acknowledged and discussed this aspect of the invention. Failure to consider the subject matter of the claim as a whole constitutes legal error. Accordingly, all rejections of claim 28 are reversed.

Claim 29 requires a product made by a specific process which is a non-glycosylated protein having a specific amino acid sequence and is substantially free of other protein. We find that the examiner has not provided adequate reasons to believe

[3] In re Best, 562 F.2d 1252, 1255, 195 USPQ 430, 433-434 (CCPA 1977).

that the reference products are identical, or substantially identical, to the product as claimed.

Hirano describes a purified product designated "BCDF" containing two bands of protein, and speculates that the difference in molecular weights "is the result of post-translational modification or the formation of breakdown products." Appellants argue that without a comparison of the amino acid sequences of these protein species it is impossible to assume that a single protein is present. The examiner argues that the specific activity is an indication of a substantially pure protein and is sufficient to identify the protein. The examiner also argues that the amino acid sequence is inherent to the protein. However, we note that the reference relied upon by the examiner identified as JP 61-115025 discusses "BCDF" and lists Hirano as an inventor. In the Japanese patent, "BCDF" was made from a similar cell line and purified by the same procedure as reported in Hirano. The N-terminal sequence of "BCDF" is reported as
Pro Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala which lacks the N-terminal Ala residue recited in the claimed protein. Viewing these references together, we find that the Japanese patent provides evidence that the protein(s) of Hirano do not necessarily meet the particular limitations of claim 29. In our view, these circumstances are such that the examiner needed to explain the discrepancy between the respective amino acid sequences and explain how Hirano suggests the particular claimed product. The examiner did not do so.

In regard to Weissenbach, we note that the reference teaches a method of inducing and fractionating mRNAs encoding an interferon activity, making a cDNA from the fractionated mRNA, partial cDNAs (of undisclosed sequence) encoding "Hu IFN-β2", a specific mRNA hybrid-selected by the cDNA, and an isolated protein made by in vitro translation of the mRNA (Figure 4, lanes 7-9). In vitro translation products are the only proteins disclosed in this publication. See figures 2 and 4. The translation products are not purified free of other proteins; at best, they are immunoprecipitated. It is not clear to us, and the examiner has not explained, how the translation products disclosed in this publication meet the claim limitation of "substantially free of other protein".

In regard to Zilberstein, the reference mentions a biochemical fractionation procedure which yields "IFN-β2" and preparation of antisera. However, the publication refers to an unpublished document for details. Thus, it is not clear whether the reference enables the fractionation procedure[4]. The reference also discloses a protein made by rodent cell lines transfected by human genes. Being rodent cells, the cells would not

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[4] See In re Payne, 606 F.2d 303, 314, 203 USPQ 245, 255 (CCPA 1979) ("References relied upon to support a rejection under 35 U.S.C. § 103 must provide an enabling disclosure, i.e., they must place the claimed invention in the possession of the public. In re Brown, 51 CCPA 1254, 1259, 329 F.2d 1006, 1011, 141 USPQ 245, 249 (1964). An invention is not 'possessed' absent some known or obvious way to make it. In re Hoeksema, 55 CCPA 1493, 1500, 399 F.2d [sic 269]. 274, 158 USPQ 596, 601 (1968).") See also In re Vaeck, 947 F.2d 488, 495, 20 USPQ2d 1438, 1444 (Fed. Cir. 1991) (To be enabling a disclosure must teach persons skilled in the art to make and use the claimed invention without undue experimentation.).

produce the nonglycosylated protein required by the claim. Thus, absent a fact-based explanation from the examiner, we conclude that the proteins disclosed in this publication do not meet the claim limitations.

In sum, we do not find that the examiner has provided sufficient reason to believe that any of the reference products are identical, or substantially identical, to the product of claim 29. Therefore we reverse this rejection.

Rejection 2

Claims 28 and 29 stand rejected as obvious over Hirano, Weissenbach, Zilberstein, Revel, or JP 115025A in view of Gilbert, Ingolia, or Clark. The examiner states that Hirano, Weissenbach, Zilberstein, and Revel do not specifically teach the amino acid sequence of the protein, and with the exception of Revel do not specifically teach the expression of IL-6 in a prokaryotic cell or the production of an nonglycosylated IL-6. The JP document discloses a purified protein, and an N-terminal sequence. The examiner argues that it would have been obvious to use the methods of Gilbert, Ingolia, and Clark for expression of an nonglycosylated protein, for the advantages of homogeneity, ease of handling, and low cost. We disagree.

In discussing obviousness in In re O'Farrell, 853 F.2d 894, 903-04, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988)(citations omitted):

> The admonition that "obvious to try" is not the standard under § 103 has been directed mainly at two kinds of error. In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gate either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. . . . In other, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

In order to succeed in producing a protein by expression in a prokaryotic cell, one must necessarily obtain a nucleic acid that encodes the desired protein. Hirano provides no guidance in this regard. While JP 115025 does describe the amino acid sequence for a small segment of the desired protein, the N-terminal sequence is not the same as the N-terminal sequence of the product recited in the claims. Even if one of skill in the art were to succeed in obtaining a coding sequence based upon the information given, the examiner has not explained why it would have been obvious to add an alanine residue at the N-terminus of the sequence disclosed in the patent.

In contrast, Weissenbach, Zilberstein, and Revel do teach nucleic acids. Weissenbach teaches sucrose gradient fractionation of mRNA from superinduced fibroblasts, isolation of about 25 cDNA clones, selection of one clone A341, identification of 12 of the clones as having overlapping sequence, and identification of an mRNA of

1270+/-70 nucleotides as hybridizing to clone A341. Zilberstein in addition provides a restriction map for two genomic clones. Revel discusses how one would produce a cDNA, and mentions the desirability of bacterial expression. The examiner argues that Weissenbach and Zilberstein set forth sufficient information/direction to obtain the nucleotide sequence for the IFN-β2 DNA. However, these references provide little or no guidance related to the existence or nonexistence of a secretory leader sequence, or production of a mature protein. We note that the claims are not directed to IL-6 protein as it is encoded by the mRNA, but require the protein divested of its leader secretory sequence (see specification pages 7-8 and Figure 1). Even if these references provided sufficient guidance to produce full-length cDNAs and express the products encoded by the cDNAs, they provide no guidance or direction to the claimed product, which is lacking a signal sequence. Therefore combination of the teachings of these references with Gilbert, Ingolia or Clark would not lead to the claimed product. The only art teaching a particular N-terminal protein sequence is JP 115025A. This reference does not teach or suggest the same N-terminus as recited in the claim, and furthermore does not provide any reason to combine its teachings regarding a B-cell differentiation factor protein with the teachings of the interferon nucleic acids.

Therefore, we conclude that the cited references do not provide sufficient guidance or direction to lead one of ordinary skill in the art to the product as claimed, with a reasonable expectation of success.

REVERSED

William F. Smith Administrative Patent Judge	))))) BOARD OF PATENT
Demetra J. Mills Administrative Patent Judge	)) APPEALS AND)) INTERFERENCES
Eric Grimes Administrative Patent Judge	))

Appeal No. 2001-2308
Application 07/704,578

WS/dm

Margaret A. Boulware
Jenkens & Gilchrist
1100 Louisiana Street
Suite 1800
Houston, TX 77002-5214

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 33

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte MARTIN COLE, THOMAS T. HOWARTH and CHRISTOPHER READING

Appeal No. 99-0624
Application 07/749,482[1]

HEARD: May 3, 1999

Before GARRIS, OWENS and LIEBERMAN, *Administrative Patent Judges*.

OWENS, *Administrative Patent Judge*.

DECISION ON APPEAL

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[1] Application for patent filed August 15, 1991. According to the appellants, the application is a continuation of Application 07/210,339, filed June 23, 1988, now abandoned; which is a continuation of Application 05/569,007, filed April 17, 1975, now abandoned.

This is an appeal from the examiner's final rejection of claims 112-118, 120-122 and 128-131, which are all of the claims remaining in the application.

THE INVENTION

Appellants' claimed invention is directed toward pharmaceutical compositions which are useful for β-lactamase inhibition in humans and animals and which include clavulanic acid or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier or in pharmaceutically acceptable form. Claims 112 and 128 are illustrative and read as follows:

112. A pharmaceutical composition useful for effecting β-lactamase inhibition in humans and animals which comprises a β-lactamase inhibitory amount of a pharmaceutically acceptable salt of clavulanic acid, in combination with a pharmaceutically acceptable carrier.

128. A β-lactamase inhibitory pharmaceutical composition comprising solid clavulanic acid or a pharmaceutically acceptable salt thereof in a β-lactamase inhibitory amount in pharmaceutically acceptable form.

THE REFERENCE

Eli Lilly & Co. (Lilly) 1,315,177 Apr. 26, 1973

THE REJECTIONS

Claims 112-118, 120-122 and 128-131 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Lilly, and under 35 U.S.C. § 103 as being obvious over Lilly.

Rejection under 35 U.S.C. § 102(b)

In order for a claimed invention to be anticipated under 35 U.S.C. § 102(b), all of the elements of the claim must be found in one reference. *See Scripps Clinic & Research Found. v. Genentech Inc.*, 927 F.2d 1565, 1576, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991).

The examiner argues that clavulanic acid inherently was produced and isolated by Lilly (answer, pages 3-24). This argument is deficient in that it does not address the limitation in each of the independent claims which requires that either clavulanic acid (claim 112) or a pharmaceutically acceptable salt thereof (claims 113 and 120) be in combination

with a pharmaceutically acceptable carrier, or be in a pharmaceutically acceptable form (claim 128), and it is not apparent where Lilly discloses each of these limitations. We therefore do not sustain the rejection under 35 U.S.C. § 102(b).

Rejection under 35 U.S.C. § 103

The examiner argues that since Lilly's "other antibiotic substances" have been found to include clavulanates, it would have been *prima facie* obvious to one of ordinary skill in the art to purify the clavulanates and use them in conventional forms for administration (answer, pages 24-25). This argument is not well taken because the examiner has not established that it was known in the art that Lilly's "other antibiotic substances" include clavulanic acid or clavulanates. The examiner argues that Lilly's characterization of the antibiotic substances as such indicates that the substances were separated and tested sufficiently to determine that they are antibiotics and include clavulanic acid and clavulanates

(answer, page 6). This argument is not persuasive because it is based purely on speculation, and such speculation is not a sufficient basis for a *prima facie* case of obviousness. *See In re Warner*, 379 F.2d 1011, 1017, 154 USPQ 173, 178 (CCPA 1967), *cert. denied*, 389 U.S. 1057 (1968); *In re Sporck*, 301 F.2d 686, 690, 133 USPQ 360, 364 (CCPA 1962). Hence, we do not sustain the rejection under 35 U.S.C. § 103.

DECISION

The rejections of claims 112-118, 120-122 and 128-131 under 35 U.S.C. § 102(b) as being anticipated by Lilly, and under 35 U.S.C. § 103 as being obvious over Lilly, are reversed.

REVERSED

BRADLEY R. GARRIS Administrative Patent Judge	)	
TERRY J. OWENS Administrative Patent Judge	)	BOARD OF PATENT APPEALS AND INTERFERENCES
PAUL LIEBERMAN Administrative Patent Judge	)	

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Paper No. 35

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte YUPIN CHAROENVIT, STEPHEN L. HOFFMAN,
RICHARD L. BEAUDOIN, DECEASED, BY BARBARA A. BEAUDOIN

Appeal No. 1999-1413
Application No. 08/176,024

ON BRIEF

Before SCHEINER, MILLS and GRIMES, Administrative Patent Judges.

MILLS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the final rejection of claims 1 through 7, 11, and 12, which are all of the claims pending in the application.

Claims 1, 4, and 11 are representative and read as follows:

1. A formulation protective against Plasmodium vivax for a time commensurate with the time monoclonal antibody Navy Vivax Sporozoite 3 (HB10615) remains at pharmacologically active levels in a subject's blood stream, comprising a pharmaceutical amount sufficient to provide passive immunization of Navy Vivax Sporozoite 3 (HB10615) in a pharmaceutically suitable injectable solution.

4. A method of providing protection from Plasmodium vivax induced malaria for subjects experiencing exposure to infected mosquitoes, for a time commensurate with the time monoclonal antibody Navy Vivax Sporozoite 3 (HB 10615) remains at pharmacologically active levels in a subject's blood stream, that comprises introducing and circulating the antibody Navy Vivax Sporozoite 3 (HB 10615) in the subject's blood stream.

11. A humanized antibody capable of providing passive protection against Plasmodium vivax wherein said antibody has a variable region comprising the hyper variable regions of the heavy and light chains of monoclonal antibody Navy Sporozoite 3 (HB10615) and human antibody framework regions.

The examiner relies on the following references:

McCutchan et al (McCutchan 1) 4,694,944 Sept. 15, 1987

McCutchan, T.F. et al (McCutchan 2). "Sequence of the Immunodominant Epitope for the Surface Protein Sporozoites of Plasmodium vivax," Science, Vol. 23, pp. 1381-1383 (1985)

Harlow et al. (Harlow), Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory pp. 287 (1988)

Charoenvit, Y. et al. (Charoenvit), "Inability of Malaria Vaccine to Induce Antibodies to a Protective Epitope Within its Sequence," Science, Vol. 251, pp. 668-671 (1991)

Harris et al. (Harris), "Therapeutic Antibodies - The Coming of Age," Tibtech, Vol. 11, pp. 42-44 (1993)

Mitchell, G. H., (Mitchell), "An Update on Candidate Malaria Vaccines," Parasitology, Vol. 98, New York, pp. S29-S46 (1989)

Grounds of Rejection

1. Claims 1-3 stand rejected under 35 U.S.C. §103. As evidence of obviousness, the examiner cites McCutchan (1 and 2) and Harlow.

2. Claims 1-7, 11 and 12 stand rejected under 35 U.S.C. §112, first paragraph. As evidence of nonenablement, the examiner cites Charoenvit, Harris, and Mitchell.

We reverse both rejections.

DISCUSSION

Procedural Matters

In this case, an Appeal Brief with four attached 1.132 declarations was filed concurrent with a proposed amendment, on March 1, 1996. After several interviews and written communications, amended claims were entered by the Examiner, the effect of amendment entry on the rejections of record was communicated to the appellant on August 21, 1996, and a Substitute Brief was filed September 20, 1996, containing arguments directed to the amended claims. The Substitute Brief also refers to the

declarations by Drs. Steven L. Hoffman (1st and 2nd declarations), Yupin Charoenvit, and Thomas F. McCutchan, which were attached to the original Brief.

In the Examiner's Answer, four rejections under 35 U.S.C. § 103 were withdrawn. No new grounds of rejection were made, and no Reply Brief was filed.

Background

Plasmodium vivax is one of the four species of parasite causing malaria in humans (specification, page 1). Despite major efforts over at least 20 years, a commercially viable malaria vaccine has not been achieved (page 2 of the December 28, 1993 amendment to the specification). The present invention involves a monoclonal antibody, here designated NVS3. The monoclonal antibody has been described in the prior art (specification, page 2). This antibody binds to an epitope within a repeated nine amino acid sequence of the circumsporozoite protein of P. vivax (specification, page 8). Prior to the invention, recombinant proteins comprising the P. vivax repeated amino acid sequence failed to induce a significant protective effect in Saimiri monkeys in active immunization experiments (specification, pages 3-4). An object of this invention is to provide passive protection against P. vivax by administering the antibody to a subject, where the antibodies

bind to P. vivax sporozoites in the circulation of the host and render the sporozoites noninfectious thereby preventing malarial disease (specification, pages 4 and 7-8).

Enablement

Claims 1-7, 11 and 12 stand rejected under 35 U.S.C. §112, first paragraph. As evidence of nonenablement, the examiner cites Charoenvit, Harris, and Mitchell.

Although not explicitly stated in section 112, to be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without "undue experimentation." In re Wands, 858 F.2d 73, 736-37, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988); In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) (the first paragraph of section 112 requires that the scope of protection sought in a claim bear a reasonable correlation to the scope of enablement provided by the specification). Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. In re Marzocchi, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971).

An analysis of whether the claims under appeal are supported by an enabling disclosure requires a determination of whether that disclosure contained sufficient information regarding the subject matter of the appealed claims as to enable one skilled in the pertinent art to make and use the claimed invention. In order to establish a prima facie

case of lack of enablement, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. See In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure). See also In re Morehouse, 545 F2d 162, 165, 192 USPQ 29, 32 (CCPA 1976). The threshold step in resolving this issue is to determine whether the examiner has met his burden of proof by advancing acceptable reasoning inconsistent with enablement.

Factors to be considered by the examiner in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman, [230 USPQ 546, 547 (Bd Pat App Int 1986)]. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

In the present case, the examiner cites the state of the art and the lack of working examples involving humans as the factors leading to a conclusion of non-enablement. Specifically, the examiner argues (Answer, page 6):

> The state of the art to which the invention pertains is such that as of this date passive immunization has not been used to prevent malaria in humans and that there are no vaccines for active or passive immunization that are accepted as being effective for prevention of P. vivax malaria. Charoenvit et al. (Science 251) states that it has never been definitively established in humans that circulating antibodies to the sporozoite of Plasmodium can prevent infection. Furthermore, Harris et al. establishes the use of monoclonal antibodies for in vivo human therapy is art-recognized to be highly experimental and unpredictable to those of skill in the art. The record contains no working examples relating to the use of the NVS3 antibody for treatment of P. vivax malaria in humans....
>
> The invention has been exemplified using the monkey model. However, the evidence obtained using the monkey model is not sufficient to allow one of ordinary skill in the art to predict the ability to practice the claimed invention for treatment of humans given that the monkey model used to exemplify the claimed invention is not an art-accepted model which is recognized as having a clear correlation with human efficacy for the evaluation of agents for passive immunotherapy of malaria.

On the other hand, the appellants argue that proof of efficacy in humans is not required, and that the monkey animal model tests disclosed in the specification are accepted by experts in the field. Substitute Brief, pages 13-15.

The specification provides a working example demonstrating efficacy of the claimed formulation in a nonhuman primate, the Saimiri monkey. Example 3, pages 13-15. In addition, the Hoffman Declaration of record provides an expert opinion that "most experts in the field consider this monkey model to be the most reliable system for predicting what will occur in humans." Hoffman Declaration, page 6. The Hoffman Declaration also cites long-held knowledge in the art of passive immunotherapy for acute malaria in human children. Hoffman Declaration, pages 4-5.

Although the examiner considered several scientifically conservative statements regarding the acceptability of the animal model of record, such as, "this monkey model system has not been validated" (Hoffman declaration, page 6), and "[w]ith the exception of the work carried out in man, the validity of all the experimental systems is open to challenge" (Mitchell, page 2), we do not find that the examiner has reviewed the evidence of enablement provided by appellants as a whole.

The cases of In re Fouche, 439 F.2d 1237, 1243, 169 USPQ 429, 434 (CCPA 1971) and In re Brana, 51 F.3d 1560, 1563, 34 USPQ2d 1436, 1439 (Fed. Cir. 1995), recognize that 35 U.S.C. §101 rejections for utility present similar issues as 35 U.S.C. §112 rejections for nonenablement. Thus, it is appropriate to consider relevant utility case law to the present enablement issue.

In Brana, the Federal Circuit stated, "Our court's predecessor has determined that proof of an alleged pharmaceutical property for a compound by statistically significant tests with standard experimental animals is sufficient to establish utility." In re Brana, 51 F.3d 1560, 1567, 34 USPQ2d 1436, 1442 (Fed. Cir. 1995); In re Krimmel, 292 F.2d 948, 953, 130 USPQ 215, 219 (CCPA 1961). In addition, "...pharmacological testing of animals is a screening procedure for testing new drugs for practical utility." Cross v. Iizuka, 753 F.2d 1040, 1051, 224 USPQ 739, 747 (Fed. Cir. 1985); In re Jolles, 628 F.2d 1324, 1327, 206 USPQ 885, 890 (CCPA 1980).

It is appellants' position that successful in vivo testing for a particular pharmacological activity in an art accepted model (monkeys) establishes a significant probability that in vivo testing for this particular pharmacological activity will be successful in humans. On the facts before us, we agree.

Appellants submit that they have provided evidence of efficacy of the claimed formulation protective against Plasmodium vivax in the most reliable and standard animal model accepted by experts in the field for predicting the likelihood of success of the claimed invention in humans. Substitute Brief, page 13.

Based upon the relevant evidence as a whole, we find there to be a reasonable correlation between the disclosed in vivo utility and an in vivo activity in humans, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence. Compare Cross v. Iizuka, 753 F.2d 1040, 224 USPQ 739 (Fed. Cir. 1985); Nelson v. Bowler, 626 F.2d 853, 856, 206 USPQ 881 (CCPA 1980). Therefore, we will not sustain the rejection of the claims for lack of enablement.

Obviousness

Claims 1-3 stand rejected under 35 U.S.C. §103. As evidence of obviousness, the examiner cites McCutchan (1 and 2) and Harlow.

In rejecting claims under 35 U.S.C. § 103, the examiner bears the initial burden of presenting a prima facie case of obviousness. See In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). A prima facie case of obviousness is established when the teachings from the prior art itself would appear to have suggested the claimed subject matter to a person of ordinary skill in the art. In re Bell, 991 F.2d 781, 783, 26 USPQ2d 1529, 1531 (Fed. Cir. 1993). A reference is considered in its entirety for what it fairly suggests to one skilled in the art. In re Wesslau, 353 F.2d 238, 241, 147 USPQ 391, 393 (CCPA 1965). With this as background, we analyze the prior art applied by the examiner in the rejection of the claims on appeal.

According to the examiner, McCutchan 1 and 2 describe monoclonal antibodies which are specific for epitopes of a peptide which corresponds to a region of the P. Vivax CS (circumsporozite) protein. The specification, page 2, states that the monoclonal antibody disclosed by McCutchan et al (Science 230) and McCutchan et al (U.S. Patent No. 4,693,994) is the monoclonal antibody of the instant invention which is designated NVS3. Answer, page 5. The examiner acknowledges that the McCutchan references do not teach a composition comprising a pharmaceutical amount of a monoclonal antibody NVS3 in a pharmaceutically acceptable carrier. Id.

Harlow is cited by the examiner as establishing that it was well known in the art at the time of the invention to produce solutions of monoclonal antibodies in phosphate

buffered saline (PBS) which is considered to be a pharmaceutically acceptable diluent for storage of antibodies.

The examiner summarizes (Answer, pages 5-6),

> It would have been obvious for one of ordinary skill in the art to produce solutions consisting of NVS3 monoclonal antibody as taught by McCutchan et al references. One of ordinary skill in the art would have been motivated to produce such compositions in order to form stable storage compositions, or working solutions for us in assays, etc. The antibody concentrations in such compositions would have been those which would be considered to be pharmaceutical amounts, and solutions comprising the NVS3 antibody PBS would be considered to be pharmaceutically injectable solutions given that the buffer PBS is a pharmaceutically acceptable diluent. Even though the appellants characterize the claimed formulations as being for use in passive protection against *P.vivax*, the claims read on the ingredients *per se*, which in the case of the instant claims are NVS3 antibody in a pharmaceutically acceptable carrier.

Appellants argue in response to this rejection that, at best the examiner has argued that it would be obvious to try using the NVS3 monoclonal antibody for passive immunization and that it would have some protective activity. Substitute Brief, page 24. Appellants argue the examiner has failed to provide evidence to support a reasonable expectation of the success of passive immunization using the monoclonal antibody, as claimed. Id. Furthermore, appellants argue that Harlow teaches away from the invention by recommending addition of sodium azide, a poison, as a preservative in monoclonal antibody solutions. Substitute Brief, page 32.

We agree with appellants that the examiner has failed to establish a prima facie case of obviousness on the record before us. McCutchan teaches the claimed monoclonal

antibody in the context of an analytical tool. Harlow, the secondary reference, states that when preparing a PBS solution of monoclonal antibodies in the laboratory, "[i]f there is no reason to avoid the use of sodium azide, add to 0.02%". Harlow, page 287. In our view, neither reference, however, provides any reason for one of ordinary skill in the art to avoid the use of sodium azide in preparing a monoclonal antibody solution, such as for preparing a composition for use in vivo.

The diagnostic use of a monoclonal antibody as described by McCutchan 1 and 2, in view of Harlow, would reasonably appear to have suggested that sodium azide be used in preparing such monoclonal antibody solutions. Therefore, taking the teachings of the references in their entirety, the references as a whole would have suggested to one of ordinary skill in the art a composition comprising a monoclonal antibody, PBS and sodium azide in an antibody solution, leading to a solution which is not a pharmaceutically acceptable formulation, as claimed. Moreover, we find no evidence of record suggesting the use of, or supporting a reasonable expectation of success for the use of the monoclonal antibody for preparation of a pharmaceutical formulation for passive immunization against P. vivax. Therefore, we will not sustain the rejection of the claims for obviousness.

CONCLUSION

The rejection of claims 1-3 under 35 U.S.C. §103 in view of McCutchan (1 and 2) and Harlow is reversed.

The rejection of claims 1-7, 11 and 12 under 35 U.S.C. §112, first paragraph is reversed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

REVERSED

Toni R. Scheiner Administrative Patent Judge	)
Demetra J. Mills Administrative Patent Judge	) BOARD OF PATENT) APPEALS AND) INTERFERENCES
Eric Grimes Administrative Patent Judge	)

Paper No. 26

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte THERESA M. SILER-KHODR

Appeal No. 1996-2468
Application 08/091,899

ON BRIEF

Before WINTERS, WILLIAM F. SMITH, and MILLS, Administrative Patent Judges.

MILLS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. §134 from the examiner's final rejection of claims 1 through 13, which are all of the claims pending in this application.

We reverse.

Claim 1 is illustrative of the claims on appeal and reads as follows:

1. A method for regulating placental cell production of thromboxane and $PGF_{2\alpha}$ comprising treating placental cells with a pharmacologically effective amount of insulin-like growth factor I sufficient to inhibit thromboxane and prostaglandin $F_{2\alpha}$ production without affecting prostacyclin or prostaglandin E_2 production.

References of record regarding issues of enablement include:

Vane et al. (Vane), "The contribution of prostaglandin production to contractions of the isolated uterus of the rat," Br. J. Pharmac., Vol. 48, pp. 629-39 (1973)

Zuckerman et al. (Zuckerman), "Inhibition of Human Premature Labor by Indomethacin," The American College of Obstetricians and Gynecologists, Vol. 44, No. 6, pp. 787-92 (Dec. 1974)

Johnson et al. (Johnson), "Pharmacologic control of uterine contractility: In vitro human and in vivo monkey studies," Am. J. Obstet. Gynecol., Vol. 123, No. 4, pp. 364-75 (Oct. 1975)

Demers et al. (Demers), "Placental prostaglandin levels in pre-eclampsia," Am. J. Obstet. Gynecol., Vol. 126, No. 1, pp. 137-39 (Sept. 1976)

Valenzuela et al. (Valenzuela), "Effect of pregnancy-induced hypertension upon placental prostaglandin metabolishm: Decreased prostaglandin $F_{2\alpha}$ catabolism with normal prostaglandin E_2 catabolism," Am. J. Obstet. Gynecol., pp. 255-56 (Jan. 1980).

Mäkilä et al. (Mäkilä), "Inreased Thromboxane A_2 ProductionBut Normal Prostacyclin By The Placenta In Hypertensive Pregnancies," Prostaglandins, Vol. 27, No. 1, pp. 87-95 (Jan. 1984)

Walsh, "Preeclampsia: An imbalance in placental prostacyclin and thromboxane production," Am. J. Obstet. Gynecol., Vol. 152, No. 3, pp. 335-40 (June 1985)

Murphy et al. (Murphy), "Uterine Insulin-Like Growth Factor-1: Regulation of Expression and Its Role in Estrogen-Induced Uterine Proliferation," Endocrine Reviews, Vol. 11, No. 3, pp. 443-53 (1990)

Siler-Khoder et al. (Siler-Khoder), "IGF-I Inhibits Human Placental Prostaglandin F and Thromboxane B_2 Production," Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, The 38th Annual Meeting of the Society for the Study of Reproduction, Abstract #513, p. 179 (1992)

Rall, "Oxytocin, Prostaglandins, Ergot Alkaloids, and other Drugs; Tocolytic Agents," The Clinical Use Of Drugs That Inhibit Uterine Motility, Section IX, Chapter 39, pp. 949-53 (1990)

Geisthovel et al. (Geisthovel), "Insulin-like growth factors and thecal-granulosa-cell function," Human Reproduction, Vol. 5. No. 7, pp. 785-99 (1990)

OPINION

In reaching our decision in this appeal, we have given careful consideration to the appellants' specification and claims, to the applied prior art references, and to the respective positions articulated by the appellant and the examiner.

Rather than reiterate the conflicting viewpoints advanced by the examiner and the appellant regarding the above-noted rejection, we make reference to the Examiner's Answer (Paper No. 18½, mailed October 31, 1995) for the examiner's complete reasoning in support of the rejection, and to the appellant's Brief (Paper No. 18, filed July 24, 1995) and Reply Brief (Paper No. 19, filed December 4, 1995) for the appellant's arguments thereagainst. As a consequence of our review, we make the determinations which follow.

DECISION ON APPEAL

Claims 1-13 stand rejected under 35 U.S.C. § 112, first paragraph for failing to teach how to use the claimed invention.[1]

The examiner has admitted on the record that utility of the claimed invention has been established. Examiner's Answer, page 5. Therefore, we interpret the examiner's position of lack of enablement to be that there are no concerns of inoperability or utility,

[1] A rejection under 35 U.S.C. § 101 has been withdrawn. Examiner's Answer, page 5.

however, the specification does not teach "how to use" the claimed invention within the entire claim scope. We limit our review to the question of whether the specification teaches how to use the invention within the scope of the claims.

An analysis of whether the claims under appeal are supported by an enabling disclosure requires a determination of whether that disclosure contained sufficient information regarding the subject matter of the appealed claims as to enable one skilled in the pertinent art to make and use the claimed invention. The first paragraph of Section 112 requires that the scope of protection sought in a claim bear a reasonable correlation to the scope of enablement provided by the specification. Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970); In re Marzocchi, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971).

In order to establish a prima facie case of non-enablement, the examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure. See In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). A disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of

35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. See In re Marzocchi, 439 F.2d at 224, 169 USPQ at 370 (CCPA 1971). As stated by the court,

> it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

The threshold step in resolving this issue is to determine whether the examiner has met his burden of proof by advancing acceptable reasoning inconsistent with enablement.

It is the examiner's position that the claims, as written encompass both in vitro and in vivo methods. The examiner alleges that "the specification does not set forth any use for the in vitro methods." Examiner's Answer, page 8. The examiner admits that the "specification enables the in vitro administration of IGF-1 to placental tissue and demonstrates the changes in thromboxane and $PGF_{2\alpha}$, [but] the specification does not tell how to use the in vitro methods in a patentable manner." Examiner's Answer, page 8. The examiner further argues that the in vitro test results are "deemed to provide information for further scientific research in this area, particularly in vivo, but do not enable using IGF-I in vivo to inhibit labor or inhibit placental cell production of thromboxane and $PGF_{2\alpha}$ in vivo." Examiner's Answer, page 9. Thus, the examiner appears ultimately to argue that the in

vivo method is not enabled, i.e., the specification fails to teach the "how to use" component of the in vivo method because the in vitro model is not predictive of in vivo activity.

The examiner's first point of contention is that "the specification does not establish that the placental perifusion [sic] model is representative of the effects of IGF-I in vivo." Examiner's Answer, page 10. The examiner states that the placental perfusion model does not address the role of insulin-like growth factor binding proteins (IGF-BP) that would have been known to modulate the activity of IGF-I in vivo. Examiner's Answer, page 10 and Geisthovel. The examiner supposes that IGF-I would have been known to exert a variety of other biological effects in vivo not accounted for in the placental perfusion model. In our opinion, the examiner raises legitimate issues with respect to the predictive ability of the placental perfusion model and arguably presents a prima facie case of lack of enablement.

Once the examiner has established a reasonable basis to question the enablement provided for the claimed invention, the burden falls on the appellant to present persuasive arguments, supported by suitable proofs where necessary, that one skilled in the art would be able to make and use the claimed invention using the disclosure as a guide. See In re Brandstadter, 484 F.2d 1395, 1406, 179 USPQ 286, 294 (CCPA 1973).

The appellant responds to the argument that the placental perfusion model is not representative of the effects of IGF-I in vivo, with the submission of four publications which the appellant indicates are evidence of the acceptability of the in vitro placental perfusion

model as representative of in vivo results to those of ordinary skill in the art. In particular, the appellant argues that Walsh establishes the acceptability of the in vitro placental perfusion model as a predictor of in vivo activity, in a similar context. Walsh discloses the use of the placental perfusion model to establish the ability of indomethacin to inhibit thromboxane. (Indomethacin has been shown and is known to inhibit $PGF_{2\alpha}$ and premature labor in vivo. See Zuckerman.) Demers evidences the use of the placental perfusion model to show increased levels of PGF are associated with pre-eclampsia. Demers, Figure 2. Valenzuela evidences the use of the placental perfusion model to show decreased metabolism of $PGF_{2\alpha}$ by placental tissue is associated with toxemia. Valenzuela, Table II.

The examiner takes issue with several of the publications submitted to support the predictive value of the placental perfusion model, indicating some differences exist between the performance steps of the placental perfusion model in the references and the placental perfusion model as used in the specification. These differences, however, fail to negate the evidenced acceptability and routine use of the placental perfusion model by those of ordinary skill in the art. In addition, it appears clear from the record that there are constraints, and legal and ethical considerations which prohibit scientific experimentation directly on pregnant humans. Appellant believes that the best possible system available for demonstrating the claimed method, the human placental perfusion model has been used. Appeal Brief, page 27.

Moreover, the appellant submits Zuckerman to show that indomethacin has been shown to inhibit the activity of prostaglandins and stop uterine contractions in in vivo clinical trials of women in premature labor. It is argued that in view of "the demonstrated effect of IGF-I on placental production of prostaglandin $PGF_{2\alpha}$ and thromboxane, in view of the art recognized action of $PGF_{2\alpha}$ for labor regulation previously described using indomethacin, establish a reasonable correlation between the use of IGF-I and the inhibition of labor." Brief, page 27.

The examiner responds to this argument, arguing indomethacin has a different pharmacologic profile than indomethacin. We believe, however, that the evidence of record adequately supports agreement in activity of IGF-I and indomethacin, at least with respect to inhibition of prostaglandin and thromboxane.

We also find there to be literal support in the specification for the in vivo "how to use" requirement. Example 7 of the specification demonstrates "that IGF-I specifically inhibits vasoconstrictive prostanoid production by human placental explants in a dose related manner, and that the active doses are well within the physiological range. Therefore, appropriate doses of IGF-I may be determined for human use in the inhibition of labor using standard pharmacological parameters known to those of skill in the art to provide the described inhibition of thromboxane and prostaglandin $F_{2\alpha}$ by placenta in vivo." Specification, p. 51. Appellant appears to have adequately demonstrated extrapolation of the in vitro model to in vivo use as it pertains to

prostaglandin inhibition. This showing is uncontroverted by the examiner. Thus, it would reasonably appear that the "how to use" requirement of 35 U.S.C. § 112, first paragraph is satisfied by the above disclosure.

In vitro results with respect to the particular pharmacological activity are generally predictive of in vivo test results, i.e., there is a reasonable correlation therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are. It is not urged, that there is an invariable exact correlation between in vitro test results and in vivo test results. Cross v. Iizuka, 753 F.2d 1040, 1044, 224 USPQ 739, 742 (Fed. Cir. 1985); Nelson v. Bowler, 626 F.2d 853, 856,
206 USPQ 881, 883 (1980). It is appellant's position that successful in vitro testing for a particular pharmacological activity establishes a significant probability that in vivo testing for this particular pharmacological activity will be successful. On the facts before us, we agree.

Based upon the relevant evidence as a whole, we find there to be a reasonable correlation between the disclosed in vitro utility and an in vivo activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence. Cross v. Iizuka, supra; Nelson v. Bowler, supra.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND AND FOURTH PARAGRAPHS

Claim 2 stands rejected under 35 U.S.C. § 112, second paragraph, for failing to distinctly claim the subject matter which applicant regards as the invention. Claims 4 and 6 stand rejected under 35 U.S.C. § 112, fourth paragraph, as being in improper dependent claim form.

The appellant attempted to cancel claims 2, 4, 5, 6 and 9 in an amendment after final rejection submitted February 23, 1995, which the examiner did not enter. Appellant subsequently filed an amendment with the appeal, requesting cancellation of claims 2, 4 and 6, which the examiner did not enter.

No arguments have been presented with respect to claims 2, 4 and 6. It appears clear from the record that it is appellant's intention to cancel claims 2, 4 and 6, which would obviate the rejections of record. Until such claims are canceled, the rejections of claims 2, 4 and 6 is affirmed.

CONCLUSION

The rejection of the claims under 35 U.S.C. § 103 for obviousness is reversed. The rejections under 35 U.S.C. § 112, second and fourth paragraphs are affirmed.

REVERSED

Sherman D. Winters Administrative Patent Judge	)
William F. Smith Administrative Patent Judge	) BOARD OF PATENT) APPEALS AND) INTERFERENCES
Demetra J. Mills Administrative Patent Judge	)

Appeal No. 1996-2468
Application 08/091,899

DJM/cam

Denise L. Mayfield, Esq.
Vinson & Elkins, LLP
2300 First City Tower
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1: Am J Cardiovasc Drugs. 2001;1(3):159-66.

Gene therapy for ischemic heart disease: therapeutic potential.

Symes JF.

Division of Cardiothoracic Surgery, St. Elizabeth's Medic Center of Boston, Tufts University School of Medicine, Boston, Massachusetts, USA. james_symes@cchcs.org

Gene therapy is evolving as an alternative mode to pharmacological intervention in the treatment of cardiovascular diseases. Experimental observations indicating that introduction of genes encoding for angioge peptide growth factors could result in improvement in perfusion to ischemic myocardium have led to the initiati of a number of preliminary clinical trials to evaluate this therapeutic modality. Sustained expression of the growth factor product from somatic cells transfected with the DN for that protein has proven to be one of the major advanta of a gene therapy based approach over administration of t recombinant protein. A number of gene therapy vectors h been developed, prominent among these being adenoviral vectors and naked plasmid DNA. Whereas plasmid DNA results in less efficient transfection, its tolerability profile may be superior to adenoviral vectors. Plasmid DNA is particularly suitable when the gene product to be produce

capable of being secreted by the cell which is producing i
Vascular endothelial growth factor (VEGF) is not only essential to the process of angiogenesis, but, because it ca be secreted from intact cells, appears to be ideal for gene transfer therapy aimed at improving perfusion to ischemic myocardium. The DNA can be delivered to the myocardiu by intra-arterial or intramuscular injection. At present, dir injection into the muscle either via a small thoracotomy incision or by use of a recently developed percutaneous catheter technique appears to be superior to arterial administration. Several clinical trials based on intramyocardial injection of VEGF DNA in patients with otherwise inoperable coronary artery disease and intractat angina pectoris have recently been completed. These phas trials have documented the tolerability of gene transfer us plasmid DNA and show promise of being able to improve myocardial perfusion and reduce anginal symptoms in the majority of patients treated thus far. While the trials involving gene transfer via a thoracotomy did not allow fc randomization to a placebo group, the recent advent of a percutaneous delivery modality has allowed for randomization which should enhance our ability to determ whether angiogenic gene therapy will prove to be as effective as initial results suggest. In the future, results frc such randomized placebo-controlled trials, improvement i vectors utilized for gene transfer and innovative new delivery techniques will undoubtedly enhance the potentia of this novel approach to myocardial revascularization.

PMID: 14728030 [PubMed - in process]

1: Expert Rev Cardiovasc Ther. 2003 Jul;1 (2):215-232.

Update on gene therapy for hereditary hematological disorders.

Herzog RW, Arruda VR.

The Children's Hospital of Philadelphia, Abramson Resea Center, Rm. 310, 34th St. and Civic Center Blvd., Philadelphia, PA 19104, USA.
rwherzog@mail.med.upenn.edu

The past 3 years have been characterized by a number of impressive advances as well as setbacks in gene therapy f genetic disease. Children with X-linked severe combined immunodeficiency disorder (SCID-X1) have shown almo complete reconstitution of their immune system after receiving retrovirally transduced autologous CD34(+) hematopoietic stem cells (HSCs). However, two of 11 treated patients subsequently developed a leukemia-like disease probablydue to the undesired activation of an oncogene. Gene transfer to HSCs resulted in substantial correction of immune function and multi-lineage engraftment in two patients with adenosine deaminase (ADA)-SCID. Several Phase I clinical trials for treatment hemophilia A and B have been initiated or completed. Pai correction of hemophilia A, albeit transient, has been reported by ex vivo gene transfer to autologous fibroblast

Intramuscular injection of adeno-associated viral (AAV) vector to patients with severe hemophilia B resulted in evidence of Factor IX gene transfer to skeletal muscle anc separate trial based on hepatic infusion of AAV vector is ongoing. Sustained therapeutic levels of coagulation factc expression have been achieved in preclinical models usin retroviral, lentiviral, AAV and high capacity adenoviral vectors. Efficient lentiviral gene transfer to HSC in murin models of beta-thalassemia and sickle cell disease demonstrated sustained phenotypic correction.

PMID: 15030282 [PubMed - as supplied by publisher]

1: Gene Ther. 1998 Jan;5(1):40-9. Related Articles, l

nature publishing group

Direct intramuscular injection with recombinan AAV vectors results in sustained expression in a dog model of hemophilia.

Monahan PE, Samulski RJ, Tazelaar J, Xiao X, Nicho TC, Bellinger DA, Read MS, Walsh CE.

UNC Gene Therapy Center, University of North Carolina Chapel Hill 27599, USA.

A recombinant adeno-associated virus (rAAV) vector carrying the human factor IX cDNA was tested for safety and therapeutic gene expression in a canine model of hum hemophilia B. Intramuscular delivery of rAAV was chose based on our previous work which described long-term (> 1.5 years) reporter gene expression in immunocompetent mice following direct muscle injection. For the current stu rAAV with the human factor IX (hF.IX) cDNA under the control of the cytomegalovirus (CMV) immediate-early promoter was constructed, and rAAV/hF.IX proved capal of transducing hemophilic dog primary fibroblast cultures a dose-dependent fashion. Direct intramuscular injection 2.5 x 10(12) rAAV/hF.IX virus particles into the hindlimt of a hemophilia B dog was tolerated without bleeding or systemic reaction, and the animal was asymptomatic throughout the entire study. Transient reduction in the wh blood clotting time (WBCT) occurred during the first wee

with the anticipated development of an antihuman F.IX inhibitor antibody which corresponded with the loss of coagulation correction. At 10 weeks after vector administration, immunohistochemical analysis of injected muscle confirmed continued hF.IX expression. Limited ai of focal lymphocytic infiltration and myofiber pathology were detected which directly correlated with positive antibody staining for helper adenovirus contamination. P(tissue analysis revealed rAAV/hF.IX DNA solely in injec muscle tissue and adjacent lymph node, without dissemination to other organs (including gonads). This fir large animal study suggests that intramuscular gene deliv(using rAAV vectors is safe and supports continued development of this approach for gene therapy of human diseases, including hemophilia B.

PMID: 9536263 [PubMed - indexed for MEDLINE]

1: Gene Ther. 2004 Feb;11(3):284-91. Related Articles, l

nature publishing group

Prevention of angiogenesis by naked DNA IL-12 gene transfer: angioprevention by immunogene therapy.

Morini M, Albini A, Lorusso G, Moelling K, Lu B, Cil M, Ferrini S, Noonan DM.

Tumor Progression Section, Istituto Nazionale per la Rice sul Cancro, Largo Rosanna Benzi, Genova, Italy.

IL-12 is thought to induce a cytokine cascade with antiangiogenic effects mediated by IFN-gamma and angiostatic CXCR3 chemokine ligands. Naked DNA intramuscular injection of an expression vector plasmid producing IL-12 resulted in significant, well-tolerated elevation of serum IL-12 levels. Injection of the IL-12 plasmid at least 2 days, and up to 20 days, before subcutaneous injection of matrigel with angiogenic factor resulted in strong prevention of angiogenesis in both C57/ and nude mice. A single injection of the IL-12 plasmid contemporarily with the matrigel or 2 days after resulted i partial, statistically not significant, inhibition. Control plasmid injection did not affect either angiogenesis or angiogenesis inhibition by IL-12 protein in vivo. Angiogenesis inhibition was observed in NK cell-depleted C57/bl and nude mice as well as in IFN-gamma(-/-) and CXCR3(-/-) knockout mice, indicating that NK- and/or T-

cell-initiated IFN-gamma-chemokine cascades were not involved in the angiogenesis inhibition observed in vivo. Finally, IL-12 plasmid DNA gene transfer significantly prevented the growth and vascularization of highly angiogenic KS-Imm Kaposi's sarcoma and TS/A murine mammary carcinoma tumors in nude and/or syngeneic mi These data suggest that a preventive gene therapy approac using antiangiogenic cytokines can effectively inhibit tum angiogenesis and KS, representing an example of angioimmunoprevention.

PMID: 14737088 [PubMed - indexed for MEDLINE]

1: Gene Ther. 2004 Jan;11(2):181-6. Related Articles, l

nature publishing group

Recombinant adeno-associated virus-mediated alpha-1 antitrypsin gene therapy prevents type I diabetes in NOD mice.

Song S, Goudy K, Campbell-Thompson M, Wasserfall Scott-Jorgensen M, Wang J, Tang Q, Crawford JM, E TM, Atkinson MA, Flotte TR.

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Type I diabetes results from an autoimmune destruction o the insulin-producing pancreatic beta cells. Although the exact immunologic processes underlying this disease are unclear, increasing evidence suggests that immunosuppressive, immunoregulatory and anti-inflammatory agents can interrupt the progression of the disease. Alpha 1 antitrypsin (AAT) is a multifunctional serine proteinase inhibitor (serpin) that also displays a wi range of anti-inflammatory properties. To test the ability AAT to modulate the development of type I diabetes, we performed a series of investigations involving recombinar adeno-associated virus vector (rAAV)-mediated gene delivery of human alpha-1 antitrypsin (hAAT) to nonobes diabetic (NOD) mice. Recombinant AAV-expressing hA (rAAV2-CB-AT) was administered intramuscularly to 4-week-old female NOD mice (1 x 10(10) i.u./mouse). A

single injection of this vector reduced the intensity of insulitis, the levels of insulin autoantibodies, and the frequency of overt type I diabetes (30% (3/10) at 32 week of age versus 70% (7/10) in controls). Transgene expressi at the injection sites was confirmed by immunostaining. Interestingly, antibodies against hAAT were present in a majority of the vector-injected mice and circulating hAA was undetectable when assessed 10 weeks postinjection. This study suggests a potential therapeutic role for AAT i preventing type I diabetes as well as the ability of AAV g therapy-based approaches to ameliorate disease effectivel

PMID: 14712302 [PubMed - indexed for MEDLINE]

1: Sheng Li Xue Bao. 2003 Dec 25;55 (6):641-647.

Intramuscular injection of naked plasmid DNA encoding human preproinsulin gene in streptozotocin-diabetes mice results in a signific reduction of blood glucose level.

Wang LY, Sun W, Chen MZ, Wang X.

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The insulin complement with gene therapy has been used an experimental treatment for insulin dependent diabetes (IDDM). In the present study, we constructed naked plasn DNA vector encoding recombinant human preproinsulin gene (pCMV-IN), and injected the plasmids (100 microg/mouse) intramuscularly combined with electroporation, to achieve the in vivo transfer of insulin gene in streptozotocin (STZ)-induced diabetic C57 mice. The expression of vector-derived insulin mRNA was detected with RT-PCR in transfected local skeletal muscl The plasma insulin was elevated significantly in pCMV-I injected diabetic C57 mice, which was complemented to t level similar to the intact normal control. The protein

expression lasted for at least 35 days after the plasmid injection. Gene therapy with pCMV-IN plasmids considerably decreased the blood glucose level in STZ-induced diabetic mice from d 7 to d 35 by about 6 mmol/l The gene therapy also reduced the mortality of severe diabetic mice significantly from 100% to 37% at the 6th week. Our results indicate that the direct intramuscular injection of naked plasmids encoding human preproinsuli gene achieves the effective expression of insulin. The restoration of insulin decreases blood glucose and increas the survival in severe diabetic mice. The gene therapy mi be provided as a practical therapeutic approach to IDDM.

PMID: 14695479 [PubMed - as supplied by publisher]

1: Gene Ther. 2000 Nov;7(22):1930-9. Related Articles, L

nature publishing group

Adeno-associated virus-mediated delivery of IL- prevents collagen-induced arthritis.

Cottard V, Mulleman D, Bouille P, Mezzina M, Boissi MC, Bessis N.

UPRES EA-2361, UFR Leonard de Vinci, Universite Par XIII, Bobigny, France.

Immunomodulation of autoimmune inflammatory disease like rheumatoid arthritis can be achieved by anti-inflammatory T2 cytokines such as interleukin (IL)-4 administered by gene therapy. In this study we investigate the efficiency of adeno-associated viruses (AAV) vectors collagen-induced arthritis (CIA). After injection of AAV-LacZ in the tarsus area of mice, the expression of the transgene was localized in the deep muscles cells near the bone. LacZ expression was found in liver, heart and lung after i.m. injection of AAV-LacZ, showing a spread of the vector over the body. Anti-AAV neutralizing antibodies were detected in the serum after i.m. injection of AAV-La but they did not alter the transgene expression after re-administration of AAV-LacZ. Long-term IL-4 expression persisted 129 days after intra-muscular injection of 3.7 x (10) or 11.2 x 10(10) AAV-IL-4 p.p. (average 7.7 or 17.5 IL-4/mg proteins, respectively). More importantly, the treatment of CIA with AAV-IL-4 vector in mice produce

therapeutic benefit, since we show a diminished prevalen of the disease, a significant reduction in paw swelling, attenuated histological synovitis and a 10 days delayed or of arthritis. This is the first evidence that AAV vector-mediated gene therapy using a T2 cytokine is efficient in animal model of rheumatoid arthritis.

PMID: 11127581 [PubMed - indexed for MEDLINE]

1: Gene Ther. 2003 Sep;10(18):1543-50. Related Articles, L

nature publishing group

Protection against collagen-induced arthritis by intramuscular gene therapy with an expression plasmid for the interleukin-1 receptor antagonis

Kim JM, Jeong JG, Ho SH, Hahn W, Park EJ, Kim S, Yu SS, Lee YW, Kim S.

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The interleukin-1 receptor antagonist (IL-1Ra) is an endogenous protein that can prevent the binding of IL-1 t its cell-surface receptors. Among a number of techniques gene transfer in vivo, the direct injection of naked DNA i muscle is simple, inexpensive and safe. In this study, we evaluated the potential of intramuscular gene therapy wit plasmid DNA containing the cDNA for IL-1Ra in the prevention of murine collagen-induced arthritis (CIA). DBA/1 mice were immunized with bovine type II collage At 4 weeks after the initial immunization, expression plasmid for IL-1Ra was injected into four selected sites in the thigh and calf muscles of DBA/1 mice. Control mice received the same plasmid, but lacking the IL-1Ra coding sequence. Macroscopic analysis of paws for redness, swelling and deformities showed that the onset of modera to severe CIA in the paws of mice injected with IL-1Ra DNA was significantly prevented ($P<0.05$). In addition, b

the synovitis and the cartilage erosion in knee joints were dramatically reduced in mice treated with IL-1Ra DNA ($P<0.05$). The expression of IL-1beta was significantly decreased in the ankle joints of mice treated with IL-1Ra ($P<0.01$). Interestingly, the levels of IL-1Ra in sera and joints after intramuscular injection of IL-1Ra DNA were significantly lower than when protein had been used in previous reports, suggesting that the therapeutic effect ma be achieved by an alternative mechanism(s) rather than by systemic elevation of IL-1Ra. These observations provide the first evidence that direct intramuscular injection of expression plasmid for IL-1Ra may effectively suppress tl inflammatory pathology in arthritis.

PMID: 12907945 [PubMed - indexed for MEDLINE]

1: Zhonghua Yi Xue Za Zhi. 2002 Jul 25;82 (14):990-4.

[Effect of interleukin I receptor antigonist gene therapy on arthritis induced by type II collagen mice]

[Article in Chinese]

Yang G, Di C, Jiang M, Li F, Zhang Y, Yuan L, Song Ma D.

Department of Rheumatology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China.

OBJECTIVE: To investigate the effect of interleukin-1 receptor antagonist gene therapy on type II collagen induc arthritis in DBA/1 mice. METHODS: Plasmid pcDI-IL-1 that expresses IL-1ra in eukaryotes was constructed by inserting human IL-1ra cDNA into the eukaryotic express vector pcDI. Eukaryotes were transfected with the plasmi pcDI-IL-1ra in vivo and in vitro. The expression of 8 IL-1 was examined by ELISA and immunohistochemistry. Typ II collagen was used to induced arthritis in 32 DBA/1 mic This plasmid was injected into the muscles of DBA/1 mic with arthritis induced by type II collagen by gene gun (20 micro g/mouse) and into the muscle of 8 mice by intramuscular injection (200 micro g/mouse). After the

administration, the condition of arthritis was observed. Th serum IL-ira was examined 6 and 12 days after administration. The expression of IL-ira in muscles was tested by computerized imaging analysis. RESULTS: PCI and DNA sequencing proved the accuracy of the inserted fragment. ELISA and immunohistochemistry detected hig expression of IL-ira in vivo and in vitro. The absorbance (A) 490 value of IL-ira in the mouse muscle was 0.52 +/ 0.03 in gene gun group, and 0.48 +/- 0.02 in intramuscula injection group, all higher than that in the control group (0.41 +/- 0.02,P < 0.01 and P < 0.05). The serum IL-ira values in the gene gun group and in tramuscular injection group 6 days and 12 days after therapy were all significan higher than that in the control group (all P < 0.01; and P < 0.01 and P < 0.05). Since the 6 th day after therapy, the redness and swelling of joints in both therapies groups we alleviated. Pathological examination made 12 days after therapy showed relief at different degrees of the infiltratic of inflammatory cells, hyperplasia of synovia, bone infiltration, and cartilage destruction, especially in the ger gun group. CONCLUSION: Gene therapy of IL-ira via nc virus eukaryotic expression vactor, especially by gene gui effective in treating arthritis induced by type II collagen.

PMID: 12181096 [PubMed - indexed for MEDLINE]

1: Arthritis Rheum. 2000 Aug;43(8):1698-709.

Soluble complement receptor 1 (CD35) delivered by retrovirally infected syngeneic cells or by nak DNA injection prevents the progression of collagen-induced arthritis.

Dreja H, Annenkov A, Chernajovsky Y.

Kennedy Institute of Rheumatology, Hammersmith, and S Bartholomew's and Royal London School of Medicine an Dentistry, Queen Mary and Westfield College, UK.

OBJECTIVE: The complement system is important in the development of autoimmune inflammation, including rheumatoid arthritis (RA) and collagen-induced arthritis (CIA). Complement receptor 1 (CR1) is involved in regulation of complement activity. Studies on models of autoimmunity have demonstrated that soluble CR1 (sCR1 a potent therapeutic agent. The present study was thus undertaken to investigate the feasibility of antiinflammatc gene therapy to prevent CIA by delivery of genes encodin truncated sCR1 (tsCR1) and dimeric tsCR1-Ig. METHOL Syngeneic fibroblasts or arthritogenic splenocytes, engineered to express tsCR1 using retrovirus-mediated ge transfer, were injected into DBA/1 recipients that had bee immunized with bovine type II collagen (CII). In separate experiments, naked DNA containing tsCR1 and tsCR1-Ig

genes was injected intramuscularly into the immunized animals. The clinical development of arthritis was monitored, anti-CII levels measured, and antigenic T cell response studied. Affinity-purified tsCR1-Ig was assayed its inhibitory effect on the alternative complement pathwa in mouse serum. RESULTS: Treatment of CII-immunizec mice with the tsCR1-expressing cells inhibited developme of CIA, reduced anti-CII antibody levels, and inhibited T response to CII in vitro. Intramuscular injections of DNA encoding the CR1 genes prevented the progression of disease. Furthermore, compared with full-length sCR1, purified tsCR1-Ig was more active in inhibiting the murin alternative complement pathway. CONCLUSION: Our findings demonstrated that tsCR1 and tsCR1-Ig, when delivered via gene therapy, had a beneficial effect on autoimmune inflammation. These results indicate that targeting the complement system in RA patients may be c clinical importance.

PMID: 10943859 [PubMed - indexed for MEDLINE]

1: Hum Gene Ther. 2001 Jun 10;12(9):1063-77.

Successful gene therapy via intraarticular inject of adenovirus vector containing CTLA4IgG in a murine model of type II collagen-induced arthri

Ijima K, Murakami M, Okamoto H, Inobe M, Chikun S, Saito I, Kanegae Y, Kawaguchi Y, Kitabatake A, U T.

Division of Molecular Immunology, Institute for Genetic Medicine, Hokkaido University, 060-0815 Sapporo, Japa

We previously constructed an adenovirus vector carrying gene encoding a soluble form of fusion protein, consisting the extracellular portion of cytotoxic lymphocyte antigen (CTLA4) and the Fc portion of human immunoglobulin C (Adex1CACTLA4IgG). Murine type II collagen-induced arthritis (CIA) was treated with Adex1CACTLA4IgG. A single intraarticular injection of 1 x 10(5) PFU was able t support serum CTLA4IgG at more than 10 microg/ml for least 12 weeks and was able to inhibit the CIA clinically a histologically. In contrast, intravenous, intramuscular, or subcutaneous injection of 1 x 10(5) PFU was unable to support a significant level of serum CTLA4IgG and thus unable to inhibit the development of arthritis. Thus, we demonstrated that (1) a low-dose intraarticular injection o Adex1CACTLA4IgG was effective in delaying the onset

CIA and reducing the severity of arthritis; (2) an intraarticular (knee joint) injection of Adex1CACTLA4Ig effectively blocked the development of arthritis in distal paws; (3) the inhibitory effect of Adex1CACTLA4IgG la: at least up to 20 weeks; (4) although serum CTLA4IgG at more than 10 microg/ml persisted for at least 12 weeks, m treated by intraarticular injection of Adex1CACTLA4IgG were not anergic to adenovirus and were able to mount antibody responses against various antigens.

PMID: 11399228 [PubMed - indexed for MEDLINE]

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Mar 15 2004 17:

1: J Gene Med. 2000 Jul-Aug;2(4):222-32. Related Articles, l

Gene therapy of autoimmune diseases with vect encoding regulatory cytokines or inflammatory cytokine inhibitors.

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Gene therapy offers advantages for the immunotherapeuti delivery of cytokines or their inhibitors. After gene transf these mediators are produced at relatively constant, non-toxic levels and sometimes in a tissue-specific manner, obviating limitations of protein administration. Therapy w viral or nonviral vectors is effective in several animal mo of autoimmunity including Type 1 diabetes mellitus (DM experimental allergic encephalomyelitis (EAE), systemic lupus erythematosus (SLE), colitis, thyroiditis and variou forms of arthritis. Genes encoding transforming growth factor beta, interleukin-4 (IL-4) and IL-10 are most frequently protective. Autoimmune/ inflammatory disease are associated with excessive production of inflammatory cytokines such as IL-1, IL-12, tumor necrosis factor alpha (TNFalpha) and interferon gamma (IFNgamma). Vectors encoding inhibitors of these cytokines, such as IL-1 recep antagonist, soluble IL-1 receptors, IL-12p40, soluble TNFalpha receptors or IFNgamma-receptor/IgG-Fc fusio

proteins are protective in models of either arthritis, Type
DM, SLE or EAE. We use intramuscular injection of nak
plasmid DNA for cytokine or anticytokine therapy. Muscl
tissue is accessible, expression is usually more persistent
than elsewhere, transfection efficiency can be increased b
low-voltage in vivo electroporation, vector administration
simple and the method is inexpensive. Plasmids do not
induce neutralizing immunity allowing repeated
administration, and are suitable for the treatment of chron
immunological diseases.

Publication Types:

- Review
- Review, Tutorial

PMID: 10953913 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1996 Nov 26;93 (24):14082-7.

Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein.

Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ.

Peter Belfer Cardiac Laboratory, Johns Hopkins Universi School of Medicine, Baltimore, MD 21205, USA.

Somatic gene therapy has been proposed as a means to achieve systemic delivery of therapeutic proteins. Howev there is limited evidence that current methods of gene delivery can practically achieve this goal. In this study, w demonstrate that, following a single intramuscular administration of a recombinant adeno-associated virus (rAAV) vector containing the beta-galactosidase (AAV-lacZ) gene into adult BALB/c mice, protein expression w detected in myofibers for at least 32 weeks. A single intramuscular administration of an AAV vector containin gene for human erythropoietin (AAV-Epo) into mice resulted in dose-dependent secretion of erythropoietin an corresponding increases in red blood cell production that persisted for up to 40 weeks. Primary human myotubes transduced in vitro with the AAV-Epo vector also showe dose-dependent production of Epo. These results

demonstrate that rAAV vectors are able to transduce skel muscle and are capable of achieving sustained expression and systemic delivery of a therapeutic protein following a single intramuscular administration. Gene therapy using AAV vectors may provide a practical strategy for the treatment of inherited and acquired protein deficiencies.

PMID: 8943064 [PubMed - indexed for MEDLINE]
